

THE CHEMICAL CURE OF CLIMATIC LESIONS

Plant physiologists move into an exciting new field of research—in which climatic effects of plant growth are being chemically controlled.

by JAMES BONNER

IT APPEARS quite possible that we stand this year at the beginning of a modest new era in our understanding and control of plant growth and development. This possibility is indicated by a very small number of new facts which we have concerning the way in which plants sense and respond to temperature. These facts do not in themselves constitute a new era; actually, they constitute just a gleam in the eyes of a few people. But they have suggested a concept which will be increasingly explored in the years to come, and one which may well greatly extend our ability to cause plants to do our bidding.

An enormous amount of descriptive material concerning the effect of climate on plants has now been accumulated. We know a lot about the effects of heat and cold, the fluctuations of temperature, and other facets of climate upon crop yields and upon the ability of a particular plant to grow in a particular place. Work in the controlled-temperature conditions of the Earhart Plant Research Laboratory at Caltech has given us detailed and accurate information on the temperature optima for the growth of many species of plants, and on the way in which plant growth and yield varies with changing temperature. The facilities of the Earhart Laboratory have also made it possible to produce, at will, plants suffering from high temperature damage, from low temperature damage, and from other symptoms which plants suffer as the result of unfavorable climate.

But now that we know how to produce such climatic damage we may ask ourselves: What, actually, does a too-high or a too-low temperature do to a plant to cause such damage? What could we do about curing the symptoms?

That we ask ourselves these questions is important from the standpoint of increasing our knowledge of how

living things behave. It is important, too, from an agricultural standpoint. We know that crop yields in many regions are limited, not by average climatic conditions, but by short periods of extremes of temperature. Yields of crop plants in the cooler parts of the temperate region are said to be frequently limited by short periods of excessive temperature during the summer. Crop yields in other regions may be limited by short exposures to excessive cold. It appears quite possible that the cure or prevention of such climatic damage may be an important factor in the expansion of world food production to its ultimate limit.

Suppose that we take a particular species of plant—peas, for example. The pea plant has a relatively low optimum temperature for its growth; perhaps 20°C. Suppose we now grow plants at 20°C. and at a series of higher temperatures. At a sufficiently high temperature—about 35°C.—the plants die; the leaves become yellow, then shrivel, and after a few days the entire plant expires.

This is not due to lack of water, of light energy, or of any mineral nutrient. These are all bountifully supplied to plants at all of the temperatures. Obviously, at the higher temperatures, most of the chemical reactions which govern the plant's performance will proceed more rapidly than at lower temperatures. Chemical reactions which are bad for growth processes—those which result in the destruction of essential metabolites, for example—will go more rapidly at the higher temperature, as will the reactions of synthesis which lead to the production of these essential metabolites.

It appears that, in particular instances, damage of plants at high temperature is due to the excessive destruction of particular chemical substances. This is so in the case of the pea plant. It was shown some years ago by Arthur W. Galston, then associate professor of biology at the Institute, that pea plants may be preserved from death at high temperature by the daily application to them of the purine, adenine.

JAMES BONNER, Caltech professor of biology, by his own admission, "roams around in the fields of biochemistry and plant physiology." In the course of his roamings he has managed to do outstanding research in both fields.

It was further shown, by appropriate chemical analysis, that at higher temperatures adenine is destroyed more rapidly than it is synthesized by the pea plant. The plant therefore rapidly becomes depleted in this important metabolite. Apparently, then, the response of pea plants to excessively high temperature has a relatively simple chemical basis. Death is due to exhaustion of a particular essential compound. The plant can be kept from dying by supplying it artificially with this compound.

The behavior of the pea plant at high temperatures does not constitute an exceptional case. It has recently been found that the duck weed, *Lemna*, behaves similarly. *Lemna*, grown under appropriate conditions, fails to survive temperatures higher than about 30°C. It can, however, be kept from dying and, in fact, caused to grow very well by the application of the nucleoside, adenosine.

The implication of this experiment, too, is that, under conditions of high temperature, *Lemna* does not possess the adenosine which it requires. It would appear that the chemical defect brought about by high temperature treatment of the duck weed is limitation in adenosine supply.

How general is this simple chemical interpretation of high temperature damage to plant growth? Preliminary experiments indicate that it may be of moderately wide application, but that the critical metabolites involved in different species may be quite various. Amino acids, vitamins of the B complex, and other substances may well prove to be of critical importance in the response of still other species to high temperature.

It may be noted that the high temperature behavior of peas and *Lemna* is very similar to that of the so-called temperature mutants of *Neurospora*, which have been studied so extensively at the Institute by the microbiologically-inclined geneticists—the Neurosporologists, as they are known in Kerckhoff.

In the temperature mutants we have organisms which will grow normally at one temperature and will fail to grow at a second higher temperature. Failure to grow at the higher temperature has been shown to be due to genetically-induced inability to make one or another essential metabolite at that temperature. Temperature mutants can be cured, as it were, of their temperature sensitiveness by supplementation with the appropriate metabolite. The higher plants thus far investigated in the Earhart program are not mutants in the sense that they have been made deliberately, but what we call the normal strains of these higher plant species behave as *do temperature mutants produced by genetic machinations*.

In the past 25 years a very extensive picture has been built up, in part as a result of work done at the Institute, of the growth of the plant as controlled and integrated by a series of particular and specific chemical substances — the plant growth hormones. These substances, each made in a specific organ and transported to other organs, have to do with keeping the growth of the

various parts in tune with, and appropriate to, the growth of other parts.

The growth of small leaves into big leaves, for example, is controlled, in part, by a leaf growth hormone synthesized in the mature leaves and transported to the immature growing leaves. And for the pea plant, this leaf growth hormone has been shown to be the purine, adenine, and related substances.

Adenine is particularly limiting, then, to the growth of the leaves of the pea plant, so it is perhaps more understandable that adenine—rather than some other chemical substance—should be the one to disappear first in the leaves of the pea plant as the temperature is raised.

We know, too, a great deal about the root growth hormones, which chemically are vitamins of the B complex, and which are synthesized in mature leaves and sent down to roots, which cannot make these materials, but which need them in their growth.

Although we know a great deal about the plant growth hormones in general, we know most about those which have to do with control of the elongation of the stem—the auxins, of which indole acetic acid is the type example.

An exciting development in plant physiology during the past two years, however, has been the recognition of the fact that we really didn't know so much about stem growth as we thought we did, and that still a further substance cooperates with auxin in the control of cell elongation. This substance is gibberellin, which was discovered through the work of Japanese and British scientists, together with the work of former Caltech research fellows—Bernard O. Phinney and Anton Lang, both now at UCLA.

Gibberellin, like auxin, appears to be produced in the apical bud, according to the work of James Lockhart, a research fellow at the Institute. And gibberellin appears to be involved, too, in the response of plants to the temperatures in which they grow.

Take, for example, a biennial plant, which requires two years to complete its life cycle. Such a plant is *Hyoscyamus*, the henbane which produces, during its first season of growth, a fleshy root and a crown or rosette of leaves. Elongation of the flowering shoot fails to take place until after the *Hyoscyamus* plant has been subjected to a sufficiently long period of low temperature treatment. *Hyoscyamus* which over-winters outdoors, for example, promptly sends up a flowering shoot and flowers the following spring, when temperatures return to a sufficiently high level. *Hyoscyamus* which is kept continuously at high temperature fails to elongate its flowering shoot and does not flower.

The work of Anton Lang has shown that failure of *Hyoscyamus* to flower at high temperature is apparently due to a deficiency of gibberellin. Non-cold-treated *Hyoscyamus* plants promptly send up a flowering shoot and produce flowers if treated with minute quantities of gibberellin.

The implication of this experiment is that *Hyoscyamus*

must require particularly large amounts of gibberellin in order to elongate its flower stock, that it cannot produce such quantities of gibberellin at high temperature, but that at lower temperatures, gibberellin may be accumulated. These inferences have not yet been confirmed by appropriate chemical analysis.

In a second case, that of the biennial rye plant, Dr. Harry Highkin, of the Earhart Plant Research Laboratory, has obtained an even more complete picture of the basis of cold requirement. Biennial cereals, such as winter rye or wheat, fail to produce a shoot and do not make flowers and fruits unless they are subjected to low temperature treatment. This low temperature may be given even in the seedling stage—in which case it is referred to as a vernalization treatment.

Too simple to succeed

A great deal of effort has been expended by plant physiologists over the years in trying to discover the chemical basis of the cold requirement of cereal seedlings of the winter varieties. But all this effort was to no avail, until Highkin performed his simple and elegant experiment. Highkin's experiment is, in fact, so simple that any well informed plant physiologist would have told him beforehand that it would be doomed to failure.

Highkin took winter rye seeds, subjected to an appropriate cold treatment, and allowed these seeds to leach in water. He then removed the cold-treated seeds and placed non-cold-treated winter rye seeds in the leachate. The latter, then, soaked up into themselves all of the substances which had leaked out of the cold-treated seeds. He then planted his non-cold-treated winter rye seeds at ordinary high temperature. The plants promptly sent up shoots and flowered as though they had been, themselves, cold-treated. Substances are evidently made during cold treatment of the cold-requiring seed which can be leached out and transfused into a non-cold-treated seed, but which cause the latter seed to develop as though it had been cold-treated.

It is, of course, just a step—intellectually—to the isolation and identification of the material made in cold-requiring seeds during cold treatment. Such isolation has not been achieved however; and we do not know the chemical nature of the substance involved. But similar work with cold-requiring pea varieties has indicated that the nucleoside, guanosine, can reproduce the effects of cold treatment.

In any case, it is apparent that, in particular cases, the requirement of a plant for low temperature treatment has a chemical basis. During the low temperature treatment, the plant accumulates a particular compound which is essential to, and responsible for, the subsequent characteristic growth behavior at high temperature. In both cases, the requirements for cold treatment may be replaced by application of the appropriate chemical.

Let us now consider a case of a plant grown under temperatures sufficiently low to restrict its growth, so

that it grows more slowly than it would at optimum temperature. It was found some years ago that when the vegetative growth of *Cosmos*—an ornamental flowering plant—is restricted by low temperature, the growth rate may be increased by the addition of thiamine. Thus, for example, *Cosmos* plants were grown at a sufficiently low temperature so that their growth rate, as measured by the accumulation of dry weight, was decreased to about one half the rate characteristic of *Cosmos* plants grown at their optimum temperature. But when thiamine was applied in low concentration to the low-temperature-grown *Cosmos* plants, their growth was increased by about 50 percent; that is, growth rate was restored about half way to that characteristic of *Cosmos* plants grown at their optimum temperature.

In this case, it was possible to show by direct analysis that plants grown at the lower temperature are characterized by a lower concentration of thiamine in their tissues than is characteristic of plants grown at the optimum temperature.

Again, it would appear that the effect of low temperature in restricting growth of *Cosmos* has a relatively simple chemical basis. Although the reaction rates of many of the reactions leading to the production of the *Cosmos* plant must be decreased by low temperature, still the rate of thiamine production appears to be decreased even more than the general average. And so *Cosmos* plants which are grown at too low a temperature suffer from restriction in thiamine, and their growth rate may be increased by artificial application of this material.

Climate and chemistry

We have, then, some specific instances in which it is possible to show that climatic effects on plant growth are reducible to relatively simple chemical aberrations. These chemical aberrations can be remedied by appropriate chemical application. We do not yet know how general it may be that climatic effects are interpretable in simple chemical terms. It will be necessary to investigate this matter in a systematic fashion.

It will be necessary to find out, too, more about the chemical mechanisms involved. Why is it, for example, that adenosine is deficient in those plants grown at high temperatures? What is the enzymatic basis of this phenomenon? These are all investigatable matters.

It appears, then, that the effects of temperature on plant behavior may be mediated through chemical mechanisms. The interesting fact is that there are temperature-induced chemical defects in plant growth which can be remedied by the appropriate application of an appropriate chemical.

To forward these investigations, the Division of Natural Sciences and Agriculture of the Rockefeller Foundation has recently granted the Institute \$112,000 to be used over a five-year period, for the study of what we like to call "The chemical cure of climatic lesions of plant growth."